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IN VITRO EFFECT OF ISATIN ON WOOD DECAY FUNGI

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INTRODUCTION

Control of biodegradation is an ongoing problem in ecologically sensitive areas, requiring new approaches to deter degrading organisms. Biological control is an approach that takes advantage of the ecological relationship among organisms by using natural predators against degrading organisms, This approach has led to an interest in using predator metabolizes as pesticides.

Biological control methods offer promise as a deterrent to fungal degradation of wood by using bacteria that are natural predators of fungi (Benko, 1988a; Benko, 1988b; Benko and Highley, 1990; Croan and Highley, 1991; Dawson-Andoh and Morrell, 1990; Highley et al., 1991). Several metabolize-producing bacteria are antagonists of important wood decay fungi (Benko and Highley, 1990; Croan and Highley, 1991). Metabolizes from Streptomyces rimosus have been used to inhibit spore germination of molds and sapstain fungi, thereby preventing discoloration and deterioration of wood (Croan and Highley, 1991). Little is known about the fungitoxic properties of most bacterial metabolites.

The bacterial metabolize 2,3-indolinedione (Figure 1.) has been shown to have antifungal properties (Gil-Turnes et al., 1989). The commercial name for 2,3-indolinedione is isatin (Aldrich, Milwaukee, WI); it is produced in a cost-effective method as an intermediate in the synthesis of indigo dyes and known to have pharmacological properties (Joshi and Chand, 1982).

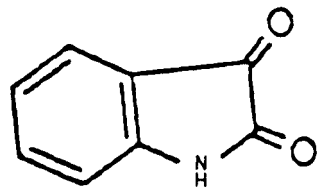

Figure 1. Molecular Structure of 2,3-Indolinedione.

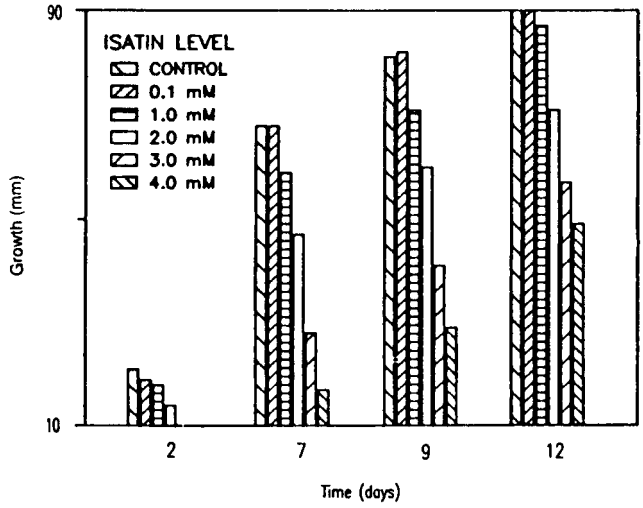

Figure 2. Effect of Isatin on Growth Rate of Postia placenta.

This paper reports the effects of isatin on white- and brown-rot wood decay fungi *in vitro* and discusses the relevance of the effects to fungal metabolism.

MATERIALS AND METHODS

Brown-rot fungi were *Postia placenta* (Fr.) M.Lars. et Lomb. (MAD-698), *Coniophora putean* (Schum.:Fr.) Karst. (MAD-515), *Gloeophyllum trabeum* (Pers. ex. Fr.) Murr. (MAD–617), *Lentinus lepideus* Fr.(MAD-534), *Antrodia carbonica* Fr. (HHB-5104), and *Fomitopsis meliae* (Underw.) Gilbn. (FP-10002-R). White-rot fungi were *Coriolus versicolor* (Mad-697), *Phanerochaete Chrysosporium* (ME-461), *Phlebia brevispora* (HHB-7030), *Ganoderma applanatum* (HHB-7823-s), *lrpex lacteus* (HHB-7328), and *Bjerkandera adusta* (L-15359).

Cultures were maintained on slants of malt extract agar (MEA) at 4C. Mycelia from slant cultures were transferred to stock culture plates containing a chemically defined, nitrogen-limited medium, mimicking nitrogen concentration in wood. The stock medium contained a previously described basal medium (Illman et al., 1989) with the addition of 1% cellobiose and 0.1 mM ammonium tartrate. Stock cultures were incubated in the dark at 27C and 70% relative humidity.

Isatin-amended medium was prepared by adding a filter-sterilized aqueous solution of isatin (Aldrich) to autoclaved, cool basal medium containing 1% cellobiose and 0.1 mM ammonium tartrate and dispensed into 100 (90 i.d.) × 20 mm sterile petri plates. Each experimental plate was inoculated by inverting a 10-mm mycelial disc from the margin of 7-day-old colonies grown on stock culture plates. Five replicate plates were prepared for each species-concentration combination. Experiments were repeated twice, Plates were incubated in the dark at 70% RH and 27C.

Diameters of colonies were measured after inoculation on days 1, 2, and 3 for the white-rot fungi *B. adusta, I. lacleus,* and *P. chrysosporium;* on days 1, 2, 3, and 6 for the white-rot fungi *C. versicolor, G. applanatum,* and *P. brevispora;* and on days 3, 6, 8, and 10 for brown-rot fungi (slower growing). Three measurements were taken of each colony and the averages compared.

To determine the concentrations of isatin to use on wood decay fungi, the effects of 0.1 to 50 mM concentrations of the chemical were tested on the growth rate of *P. placenta* (Figure 2). The experiment was repeated five times.

No growth was obtained at concentrations above 10 mM. The concentrations selected for subsequent experiments were 0, 0.1, 0.5, 1.0, and 5.0 mM.

RESULTS AND DISCUSSION

Isatin inhibited the growth rate of P. placenta at concentrations of 0.1 to 4 mM (Figure 2). The inhibition increased with increasing concentration. Growth was totally inhibited at 10 to 50 mM concentration.

The effects of isatin on growth of brown- and white-rot fungi was species-dependent with no general effect on a fungal group (Figs. 3 and 4). At the 0.1-mM concentration, isatin did not inhibit B. adusta, I. lacteus, and F. meliae during the experiments. At this concentration, isatin had no effect on G. trabeum, G. applanatum, and L. lepideus at day 3, and P. placenta after day 7; isatin had no effect on C. puteana at day 3 and stimulated growth from days 6 to 10. At concentrations >0.5 mM, isatin inhibited growth of these fungi.

Isatin stimulated the growth of the white-rot fungus P. brevispora at 0.1 to 1.0 mM, but inhibited growth at 5 mM (Fig. 4). The bacterial metabolite inhibited growth of C. versicolor, P. chrysosporium, and A. carbonica at all concentrations.

In general, the concentration of isatin had to be above 0.1 mM to inhibit growth on all fungi except P. brevispora. In future applications, a concentration between 4 and 5 mM (0.07%) and >5 mM would have to be used to be inhibitory (Figure 2). The inhibitory concentration range is similar to the 1.4-mM concentration of isatin used to inhibit growth of Lagenidium callinetes (Gil-Turnes et al., 1989).

The variable response of wood decay fungi to isatin (Figures 2, 3, and 4) is not unusual. These fungi have variable responses to several treatments, including a chitin synthetase inhibitor (Johnson, 1980; Johnson et al., 1991;) and antagonistic bacteria (Benko and Highley, 1990).

The isatin mechanism of action on wood decay fungi is not known. By using low concentrations more closely resembling physiological concentration (as opposed to toxic) to test decay fungi, we observed the dynamics of isatin effects (Figures 2, 3, and 4). At 0.1 mM, this indole was stimulatory to some wood decay fungi. Effects of 0.1, 0.5, and 1.0 mM isatin concentrations implicate a physiological role for the metabolize in growth.

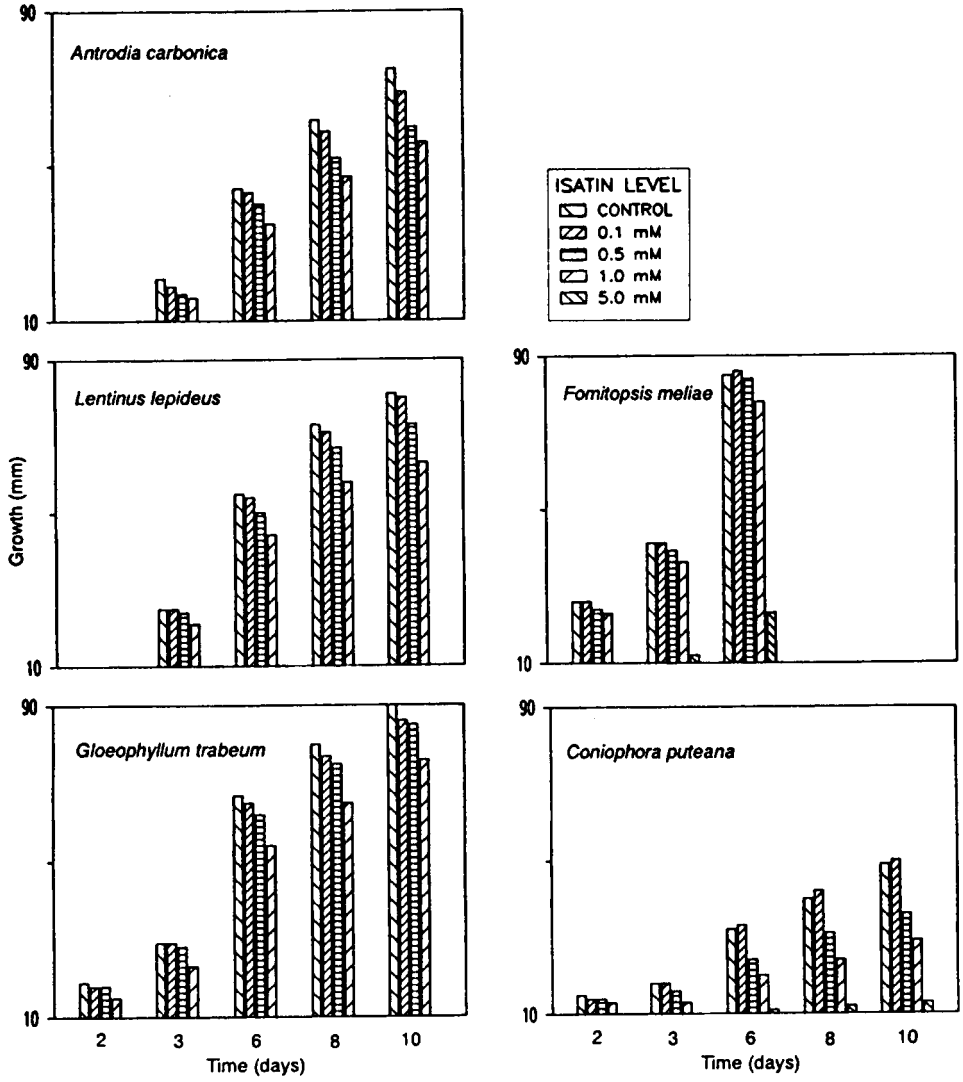

Figure 3. Effect of Isatin on Growth of Brown-rot Fungi.

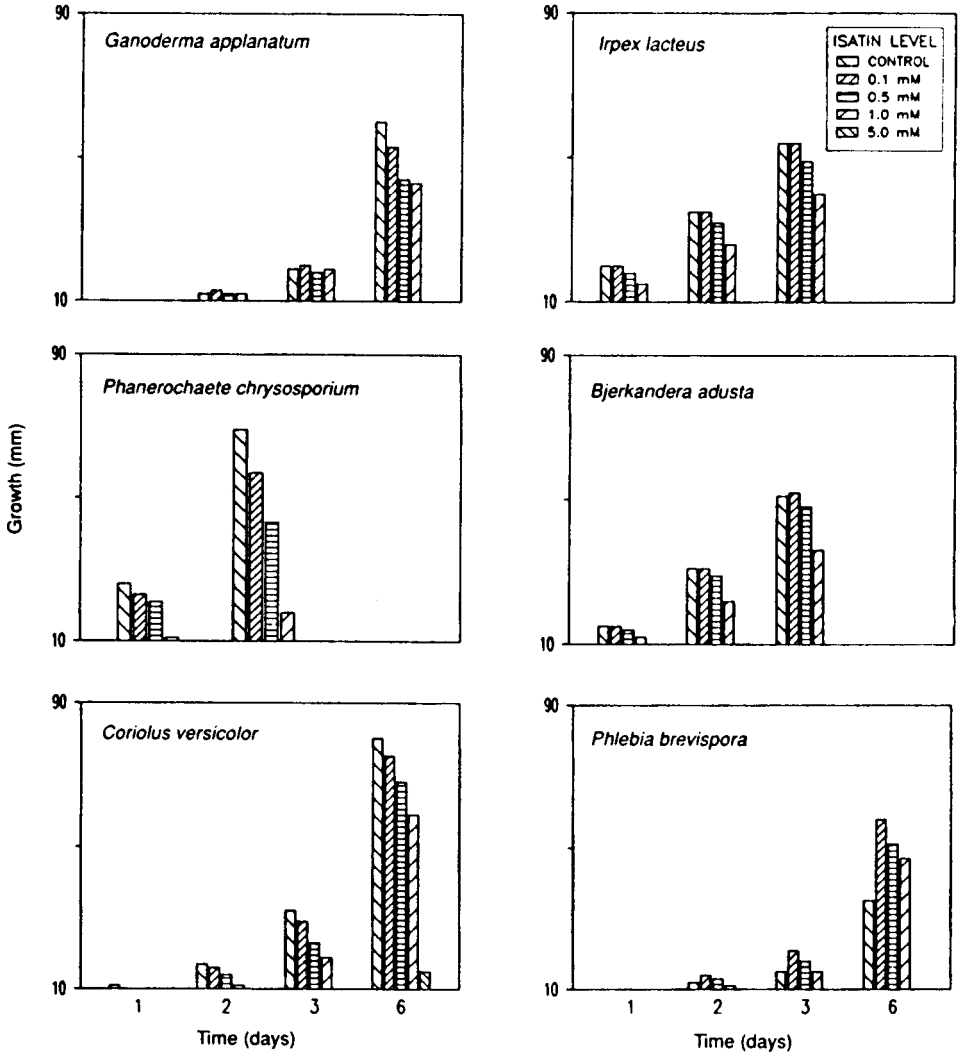

Figure 4. Effect of Isatin on Growth of White-rot Fungi.

Recent discoveries in separate scientific fields have shown that isatin has importance in ecological interactions of organisms and in mammalian physiology. Isatin has ecological significance because this indole is produced by bacteria as an antifungal metabolize (Gil–Turnes et al., 1989).

In a sequence of experiments, Gil-Turnes et al. (1989) investigated a parasitic mediation between three different organisms in an ecological interaction. A bacterial metabolize produced by Alteromonas species was shown to be toxic to the fungus L. callinetes. The bacteria were symbiotic to embryos of the shrimp Palaemon macrodactylus. The antifungal metabolize was secreted by the bacteria on the surface of the embryo, preventing colonization of the fungus and thereby protecting the susceptible embryo from a lethal bacterial infection. The bacterial metabolize was isolated and identified as 2,3-indolinedione. The mechanism of toxicity to the fungus was not studied.

Isatin was recently isolated from humans and rats (Glover et al., 1988), establishing the presence of this indole in mammalian tissues. Glover et al. identified isatin as the metabolize previously known as tribulin, a monoamine oxidase (MAO: EC.1.4.3.4) inhibitor. As an MAO inhibitor, isatin prevents oxidation of several amine substrates such as tryptamine, tyramine, and benzylamine (Zeidan, 1990). Isatin has a potent inhibitory action on MAO B, a form of the oxidase that may be an isozyme or an active form of the enzyme only under special solvent conditions. The function of isatin in mammalian physiology is not clear.

The effect of isatin on wood decay and parasitic fungi implicates its intervention in metabolic pathways responsible for fungal growth and development. The mechanism of action of isatin in mammals raises questions about fungal metabolism. Is isatin an amine oxidase inhibitor in fungi? What role would such an oxidase have on growth? A putative role for amine oxidases on growth and development would involve nitrogen availability and assimilation. To our knowledge, the effect of isatin on other amine oxidases has not been tested.

Many micro-organisms produce secondary metabolites. These allelochemicals are toxic to competitors and pathogens, protect symbiotic organisms, and thereby have an important role in ecology (Price et al., 1986). Isatin secretion by bacteria has such a role as an antibiotic.

Given the discovery that isatin is secreted by bacteria, that is is present in several mammals, and that it has at least one key function in mammalian tissue,

a search should be made for the presence and mode of action of isatin in other organisms. Isatin may well be a ubiquitous compound in organisms, serving as a modulator of amine oxidases with a role in nitrogen metabolism, availability, and cycling.

SUMMARY

The in vitro effect of the bacterial metabolize 2,3-indolinedione (isatin) was studied in 12 wood decay fungi. Results indicate that growth of all the fungi can be inhibited at concentrations above 5 mM. The effect of lower isatin concentrations on growth rate was species-dependent. At 0.5 and 1 mM, isatin inhibited growth of 11 fungi. In the white-rot fungus Phlebia brevispora, growth was stimulated at 0.5 and 1 mM. Effects of isatin implicate a physiological role for the metabolize in fungal growth.

In studies on the physiological activity of metabolizes in two unrelated areas, the chemical structure of a bacterial metabolize (unnamed) that has fungitoxic properties and that of a mammalian metabolize (tribulin) that inhibits monoamine oxidase were identified as 2,3-indolinedione. Isatin may be present in many more organisms. It is therefore relevant that isatin has been shown to have a physiological effect on wood decay fungi and that toxic levels have been identified for application. Perhaps isatin should be included in the search for microbial allelochemicals.

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